

HCO₃⁻-dependent ion transport systems and intracellular pH regulation in colonocytes from the chick

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Abstract

The current study examines the presence of the Na⁺/HCO₃⁻ cotransporter and of the Cl⁻/HCO₃⁻ exchanger in chicken colonocytes and their role in cytosolic pH (pH_i) homeostasis. pH_i was measured with 2',7'-bis(carboxyethyl)-5,6-carboxy-fluorescein (BCECF) at 25°C. Basal pH_i was 7.16 in HEPES-buffered solutions and 7.06 in those buffered with HCO₃⁻. Removal of external Cl⁻ increased pH_i and Cl⁻ reinstatement brought the pH_i towards resting values. These Cl⁻-induced pH_i changes were Na⁺-independent, inhibited by H₂-DIDS and faster in the presence than in the absence of HCO₃⁻. Cells recovered from alkaline loads by a mechanism that was Cl⁻-dependent, Na⁺-independent and inhibited by H₂-DIDS. This rate of Cl⁻-dependent cell acidification decreased as the pH_i decreased, with a Hill coefficient value close to 4. Removal of external Na⁺ decreased pH_i and readdition of Na⁺ brought pH_i towards the control values. The rate of the Na⁺-induced changes was not modified by the presence of HCO₃⁻ and was prevented by EIPA and unaffected by H₂-DIDS. In the presence of EIPA cells partially recovered from a moderate acid load only when both Na⁺ and HCO₃⁻ were present. The EIPA resistant Na⁺- and bicarbonate-dependent pH_i recovery was inhibited by H₂-DIDS and occurred at equal rates in both Cl⁻-containing and Cl⁻-free solutions. It is concluded that in chicken colonocytes bathed in HCO₃⁻-buffered solutions, both the Na⁺/H⁺ exchanger and the Cl⁻/HCO₃⁻ exchanger participate in setting the resting pH_i value. The latter transporter helps the cells to recover from alkaline loads and the first transporter, together with the Na⁺/HCO₃⁻ cotransporter, is involved in pH_i recovery from an acid load. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

The concentration of cytosolic H⁺ is finely regulated. In the short term, intracellular pH (pH_i) regulation depends on different intracellular buffers. In the

long term, pH_i is maintained by proton transport across the cell membrane [1–5]. Several studies have investigated the mechanisms involved in pH_i regulation of epithelial cells (see Ref. [3] for review). However, studies on colonocytes pH_i regulation have been scarce and limited to either culture cell lines derived from mammalian colon [6–8] or to mammalian colonic crypt cells [9,10].

Previous studies [11] designed to investigate the role of the Na⁺/H⁺ exchanger in pH_i regulation of

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colonocytes isolated from the chick were carried out in the absence of $\text{CO}_2/\text{HCO}_3^-$ to minimize the contribution of HCO_3^- -dependent mechanisms. Studies done under more physiological conditions, that is in the presence of bicarbonate, have revealed the presence in chicken colonocytes of $\text{Cl}^-/\text{HCO}_3^-$ exchange and $\text{Na}^+/\text{HCO}_3^-$ -cotransport, both involved in pH_i homeostasis.

2. Materials and methods

2.1. Solutions

Solutions composition is shown in Table 1. HCO_3^- -free solutions were titrated to $\text{pH} = 7.4$ with HEPES–Tris and equilibrated at room temperature with air. HCO_3^- -buffered solutions were equilibrated with 95% $\text{O}_2/5\%$ CO_2 to yield $\text{pH} 7.4$. In experiments with gluconate, the calcium concentration was increased to 6 mmol l^{-1} .

2.2. Intracellular pH measurements

Hubbard chickens, four- to six-weeks old, were killed by decapitation. Colonocytes were isolated by hyaluronidase incubation as described in Ref. [12]. Cell viability was assessed by determining the fraction of the cell population able to exclude 0.2% trypan blue and usually ranged from 60% to 75%.

Table 1
Composition of solutions (mM)

	A	B	C	D	E	F	G	H
Mannitol	100	100	100	260	100	100	100	240
NaCl	80	0	0	0	55	0	0	0
CaCl_2	1	0	1	0	1	0	1	0
MgCl_2	1	0	1	0	1	0	1	0
K_2HPO_4	3	3	3	3	3	3	3	3
NaHCO_3	0	0	0	0	25	25	0	0
HEPES–Tris	20	20	20	20	5	5	5	5
CholineCl	0	0	80	0	0	0	55	0
Choline HCO_3	0	0	0	0	0	0	25	25
NaGluconate	0	80	0	0	0	55	0	0
MgGluconate	0	1	0	1	0	1	0	1
CaGluconate	0	6	0	1	0	6	0	1

All solutions contained (in mM) 0.5 β -hydroxybutyrate, 10 fructose and 1 L-glutamine.

pH_i was measured fluorimetrically at 25°C with 2',7'-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF), as described in Ref. [13]. Fluorescence ratios (500/450) were correlated with pH_i at the end of each recording session by permeabilizing the cell membrane with $70 \mu\text{M}$ digitonin and constructed a calibration curve. We have previously reported for chicken colonocytes that the digitonin-based calibration, as compared to the nigericin null point procedure, underestimates the actual pH_i values by 0.15 pH units [11]. Therefore, a correction of 0.15 pH units was applied to the results.

The initial pH_i change after an experimental maneuver is defined as the change in pH_i that occurred during the first minute. Initial ion flux rates (J_{OH^-} or J_{H^+}) in nanomol per minute per milligram protein were calculated according to the formula:

$$J_{\text{ion}} = (\text{dpH}_i/\text{dt})V\beta_t$$

where V is cell volume, dpH_i/dt is the rate of change of pH_i and β_t is the total intracellular buffering capacity. β_t is the sum of the intrinsic buffer capacity (β_i) and the buffering capacity of the intracellular $\text{HCO}_3^-/\text{CO}_2$ system (β_{CO_2}), and is given by the following equation:

$$\beta_t = \beta_i + 2.3[\text{HCO}_3^-]_i$$

In HCO_3^- -free solutions, $\beta_t = \beta_i$. β_t and β_i were calculated from the increase in pH_i after the addition of 20 mmol l^{-1} NH_4Cl .

2.3. Acid-loading

For acid loading, the ammonium chloride technique was used [14]. The cells were incubated for 15 min in the Na^+ -free solutions (C or G) containing 30 mmol l^{-1} NH_4Cl . The cells were then centrifuged and resuspended in the respective NH_4Cl - and Na^+ -free solution and immediately transferred into the cuvette for the fluorescence measurement. After the fluorescence ratio had stabilized, 40 mmol l^{-1} NaCl was added to the cuvette and the rate of alkalization was determined as described above. When the effect of Cl^- -free conditions was tested the cells were preincubated for 20 min in Cl^- -free solutions (D or H). The inhibitors, if any, were already present during incubation in the Na^+ -free solutions.

2.4. Chemicals

BCECF-AM and H₂-DIDS were purchased from Molecular Probes (Eugene, OR); digitonin, hyaluronidase and all the salts used in the current study were obtained from Sigma Chemical, Madrid, Spain. 5-(*N*-ethyl-*N*-isopropyl)-amiloride (EIPA) was purchased from Merck, Sharp and Dohme. None of the chemicals used in the current work interfered with the BCECF fluorescence.

The BCECF-AM (1.45 mM), digitonin (14 mM), EIPA (4 mM) and H₂-DIDS (100 mM) were prepared in DMSO. They were stored for up to 30 days at -20°C without loss in potency. Solvent concentration did not exceed 0.5% (v:v) and did not affect the fluorescence of BCECF.

2.5. Calculations and statistics

Results are expressed as mean \pm S.E.M. Statistical significance was evaluated by the two-tailed Student's *t*-test for unpaired observations.

3. Results

3.1. Intracellular resting steady-state pH and intracellular buffering capacity of colonocytes

We have previously reported [11] that resting steady-state pH_i of chicken colonocytes, measured at 25°C , in the nominal absence of CO₂/HCO₃[−] (solu-

tion A) was 7.16 ± 0.02 ($n = 15$). The current work shows that in the presence of 25 mM HCO₃[−]/5% CO₂ (solution E) resting steady-state pH_i was 7.06 ± 0.02 ($n = 10$).

Total intracellular buffering power, β_i , calculated as described in Section 2, was 83 ± 7 mM per pH unit ($n = 10$) in the nominal absence of HCO₃[−] and 124 ± 8 mM per pH unit ($n = 10$) in the presence of 25 mM HCO₃[−]/5% CO₂. These values are higher than those reported for other cells [3,5] and close to that reported for chicken breast muscle [15].

3.2. Cl[−]/HCO₃[−] exchanger and pH_i regulation

To test whether the chicken colonocytes possess a functional Cl[−]/HCO₃[−] exchanger at resting pH_i, the transmembrane Cl[−] gradient was inverted by removal of extracellular Cl[−]. The experiments were carried out in the presence of 25 mM HCO₃[−]/5% CO₂. Cells were incubated for 15 min in Cl[−]-containing solution (solution E) and transferred to a Cl[−]-free medium (solution F) at the beginning of the fluorescence recording. This maneuver would reverse the ion gradient for Cl[−] and an operational Cl[−]/HCO₃[−] exchanger in the membrane would produce a net influx of HCO₃[−]. Fig. 1A and Table 2 show that the cells alkalized following removal of extracellular Cl[−]. This suggests that a gradient-driven efflux of chloride induced influx of base equivalents.

In another set of experiments cells were incubated for 15 min in Cl[−]-free medium (solution F) and transferred to a Cl[−]-containing medium (solution E)

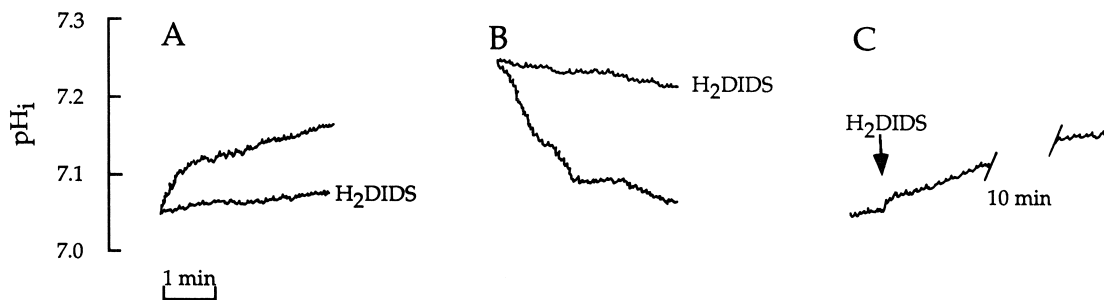


Fig. 1. Effect of H₂-DIDS and external Cl[−] on resting pH_i in isolated chicken colonocytes. (A) At the beginning of each trace, dye-loaded cells incubated for 15 min in standard solution (solution E) were suspended in Cl[−]-free solution (solution F) with or without 0.5 mM H₂-DIDS. (B) At the beginning of each trace, dye-loaded cells incubated for 15 min in Cl[−]-free solution (solution F) were suspended in Cl[−]-containing solution (solution E) with or without 0.5 mM H₂-DIDS. (C) Dye-loaded cells incubated in standard solution (solution A) were suspended in the same solution and at the time indicated by the arrow 0.5 mM H₂-DIDS was added. Each trace is representative of six independent experiments.

Table 2
Effect of external Cl^- on pH_i

Saline solution	Alkalization on Cl^- removal		Acidification on Cl^- addition	
	dpH_i/dt (U min^{-1}) $\times 10^{-2}$	J_{OH}^-	dpH_i/dt (U min^{-1}) $\times 10^{-2}$	J_{OH}^-
<i>HCO₃⁻/CO₂ buffer</i>				
Control	7 ± 0.6	32 ± 3	8 ± 1	32
With H ₂ -DIDS	$0.5 \pm 0.1^*$	$2.3 \pm 0.4^*$	$0.5 \pm 0.5^*$	$2.3 \pm 2^*$
Na ⁺ -free	6 ± 0.5	27.6 ± 2	9 ± 2	28 ± 6
<i>HEPES-buffer</i>				
Control	5 ± 1	$15.3 \pm 3^{**}$	6 ± 1	$18.4 \pm 4^{**}$

Alkalization and acidification are measured as initial rates of change in pH_i observed upon removal and reinstatement of Cl^- , respectively.

The concentration of H₂-DIDS was 0.5 mM.

The net base flux, J_{OH}^- is given in $\text{nmol min}^{-1} \text{mg}^{-1}$.

Means \pm S.E.M of six independent determinations.

* $p < 0.001$, ** $p < 0.01$, significant differences with the control in the presence of HCO_3^- (first row).

at the beginning of the fluorescence recording. Lack of chloride induced cell alkalization and reinstatement of the anion reverted the pH_i to normal values (Fig. 1B and Table 2).

The chloride-dependent pH_i changes just described were inhibited by H₂-DIDS, an inhibitor of anion exchanger in other cells [16] (Fig. 1 and Table 2).

Repetition of the experiments above described in the nominal absence of sodium (solutions G and H) led to changes in pH_i identical to those found in the presence of Na⁺ (Table 2).

In the nominal absence of HCO_3^- (solutions A and B) the chloride-dependent pH_i changes were in the same direction as those above described but they occur at a lower rate (Table 2).

All these results together suggest that chicken colonocytes present a Na⁺-independent $\text{Cl}^-/\text{HCO}_3^-$ exchanger operative under resting conditions, that can also work in HEPES-buffered solutions.

Since the other H₂-DIDS-sensitive and bicarbonate-dependent mechanisms described in the current work does not operate under resting conditions (see below), the significant cell alkalization caused by H₂-DIDS favors the view that the $\text{Cl}^-/\text{HCO}_3^-$ exchanger is functional under resting conditions (Fig. 1C).

3.3. $\text{Cl}^-/\text{HCO}_3^-$ exchanger and pH_i recovery from an alkaline load

An alkaline load was imposed by incubation of the cells in 25 mM $\text{HCO}_3^-/5\% \text{CO}_2$ (solution E) for 15

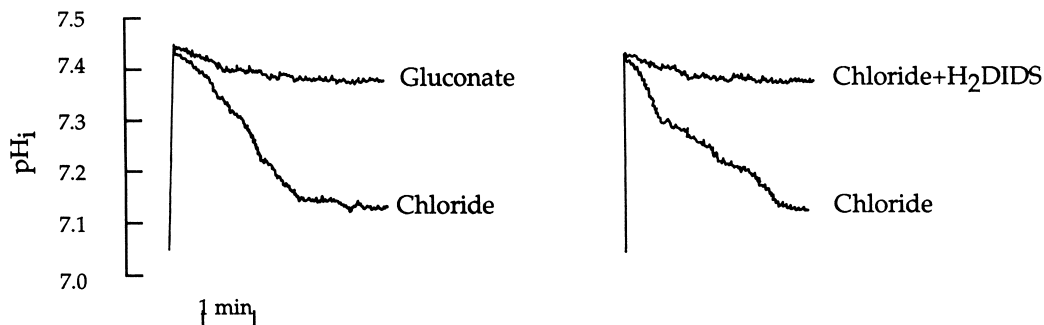


Fig. 2. External Cl^- and pH_i recovery from alkaline load. Dye-loaded cells were incubated for 15 min in the presence of 25 mM $\text{HCO}_3^-/5\% \text{CO}_2$ (solution E), with or without 0.5 mM H₂-DIDS and transferred at the beginning of the trace to HEPES-buffered solution (solution A or B) containing the indicated modifiers. The experiments were carried out in the presence of Na⁺. Each trace is representative of the number (n) of independent experiments indicated in Table 3.

Table 3

Effect of Cl^- , Na^+ and $\text{H}_2\text{-DIDS}$ (0.5 mM) on pH_i recovery from an alkaline load

Recovery solution (HEPES)	<i>n</i>	pH _i		
		At peak	1 min after peak	3 min after peak
<i>With Na⁺</i>				
Chloride	10	7.45 ± 0.02	7.31 ± 0.02	7.28 ± 0.01
Gluconate	9	7.45 ± 0.04	7.42 ± 0.01 *	7.40 ± 0.02 *
Cl [−] + H ₂ -DIDS	4	7.45 ± 0.06	7.41 ± 0.02 * *	7.38 ± 0.02 *
<i>Without Na⁺</i>				
Chloride	4	7.36 ± 0.02	7.23 ± 0.02	7.11 ± 0.02
Gluconate	4	7.34 ± 0.01	7.32 ± 0.01 * *	7.28 ± 0.02 *
Cl [−] + H ₂ -DIDS	4	7.35 ± 0.01	7.31 ± 0.01 * *	7.22 ± 0.02 *

Experimental conditions as for Fig. 2.

Means \pm S.E.M. in (*n*) independent experiments.* $p < 0.001$, ** $p < 0.01$, significant differences with its own control (first row in each case).

min and transfer to HEPES-buffered solution nominally free of CO_2 . This transfer led to an immediate alkalization (Fig. 2 and Table 3), attributed to the

rapid efflux of CO_2 , equivalent to a net proton extrusion. This alkalization was transient in the presence of chloride (transfer to solution A) and pH_i

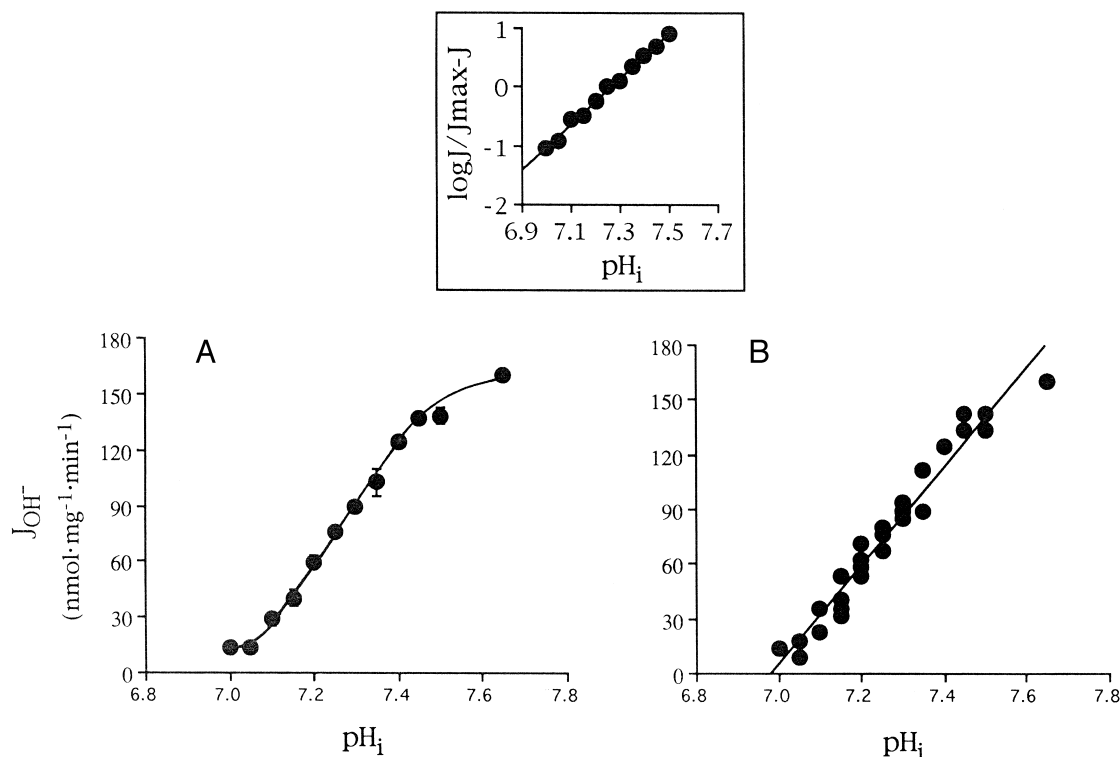


Fig. 3. Relationship between initial rate of Cl^- -dependent pH_i recovery from an alkaline load and initial pH_i . Dye-loaded cells were alkalized as described in Fig. 2. From the trace obtained in the presence of Cl^- in the recovery solution, the rate of pH_i recovery was calculated at the different pH_i values. (A) Means \pm S.E.M. ($n = 5$) of rates of OH^- efflux ($\text{nmol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$) against the means of pH_i at 0.05 pH unity intervals. (B) All the individual data are plotted and adjusted to a line by computer. Insert: Hill plot of data in (A). J_{max} was calculated from the Lineweaver-Burk plot of the data. The line was calculated by linear regression analysis, $Y = -28.27 + 3.89X$, $r = 0.99$.

decreased towards base line. This pH_i recovery was nearly abolished by H_2 -DIDS or in Cl^- -free solutions (solution B instead of A). Repetition of the experiments in the absence of Na^+ (transfer to solution C or D) did not modify the responses (Table 3). These results suggest that a Na^+ -independent, $\text{Cl}^-/\text{HCO}_3^-$ exchanger is responsible for most of the pH_i recovery from an alkaline load. We have no explanation for the residual acidification observed in the absence of Cl^- or in the presence of H_2 -DIDS. Similar acidification was observed in chicken small intestinal epithelial cells [13].

3.4. Relationship between pH_i and $\text{Cl}^-/\text{HCO}_3^-$ exchanger activity

The dependence of $\text{Cl}^-/\text{HCO}_3^-$ exchange activity on pH_i was studied in colonocytes alkalinized as described above. The results show (Fig. 3) that the activity of the exchanger increases with increasing pH_i . The relationship between net OH^- efflux ($\text{nmol mg}^{-1} \text{ min}^{-1}$) and pH_i did not follow simple Michaelis–Menten kinetics (Fig. 3). The Hill plot (Fig. 3, insert) gives an interaction coefficient of 3.89 and a $[\text{H}^+]_{0.5}$ of 54 nM. In Fig. 3B all the individual values are plotted and fitted to a line by computer. The extrapolated pH_i value at which no measurable cell acidification occurred, set-point, is approx. 6.97.

3.5. $\text{Na}^+/\text{HCO}_3^-$ cotransporter and pH_i regulation

The experiments were started with two batches of dye-loaded cells kept in HEPES- or HCO_3^- -buffered solutions on ice. After incubation of a cell sample for 15 min at 25°C in a Na^+ -free solution (solution C or G respectively of Table 1), with or without inhibitors, the cells were washed in the same solution and the sample was transferred into a cuvette of 2 ml volume for recording the fluorescence ratio. One to two min later 20 μl of 4 mol l^{-1} NaCl were added and the recording was continued for at least 3 min. Exposure of the cells to Na^+ -free solutions for 15 min, a maneuver that would reverse the ion gradient for Na^+ , acidified the cells (Table 4). Readdition of Na^+ resulted in a rapid increase in pH_i . The Na^+ -induced pH_i changes occurred nearly at equal rates in both, HCO_3^- -containing and HCO_3^- -free solutions and were prevented by EIPA and unaffected by H_2 -DIDS.

These observations indicate that in chicken colonocytes only one Na^+ -dependent alkalinizing mechanism is operative under resting conditions, the EIPA-sensitive Na^+/H^+ exchanger.

3.6. pH_i recovery from an acid load

We have previously reported [11] that in HEPES-buffered solutions (nominally $\text{CO}_2/\text{HCO}_3^-$ -free) pH_i

Table 4
Effect of Na^+ removal and readdition on pH_i

Conditions	Acidification on Na^+ removal		Alkalinization on Na^+ readdition	
	pH_i	Total acidification	dpH_i/dt (U min^{-1}) $\times 10^{-2}$	J_{H}^+
<i>HEPES buffer (reference pH_i in sol. A = 7.16 ± 0.02)</i>				
Control	6.92 ± 0.02	43 ± 5	11.0 ± 1	34 ± 3
<i>$\text{HCO}_3^-/\text{CO}_2$ buffer (reference pH_i in sol. E = 7.06 ± 0.02)</i>				
Control	6.98 ± 0.03	36.7 ± 4	10 ± 1	46 ± 6
EIPA	7.08 ± 0.05	$9.2 \pm 9^*$	$1 \pm 0.5^*$	$4.6 \pm 1^*$
H_2 -DIDS	$6.96 \pm 0.05^\#$	45.8 ± 9	10 ± 2	46 ± 6

Cells adapted to HEPES buffer or $\text{HCO}_3^-/\text{CO}_2$ buffer, that in presence of Na^+ would have $\text{pH}_i = 7.16$ or 7.06, respectively, were incubated for 15 min in Na^+ -free solutions, then transferred into the fluorimeter to measure pH_i (column 1) and to determine the change in pH_i (column 3) after adding Na^+ to a final concentration of 40 mmol l^{-1} .

Total acidification, in $\text{nmol mg protein}^{-1}$, represents the total apparent increase in cytosolic proton content following 15 min incubation in Na^+ -free conditions and it was calculated as indicated in Section 2.

Net proton efflux, J_{H}^+ , in $\text{nmol mg protein}^{-1} \text{ min}^{-1}$ was calculated as indicated in Section 2. The concentration of H_2 -DIDS was 0.5 mmol l^{-1} and that of EIPA 100 $\mu\text{mol l}^{-1}$.

Values are means \pm S.E.M. of four individual samples measured.

* $p < 0.001$ as compared with the control in the presence of bicarbonate.

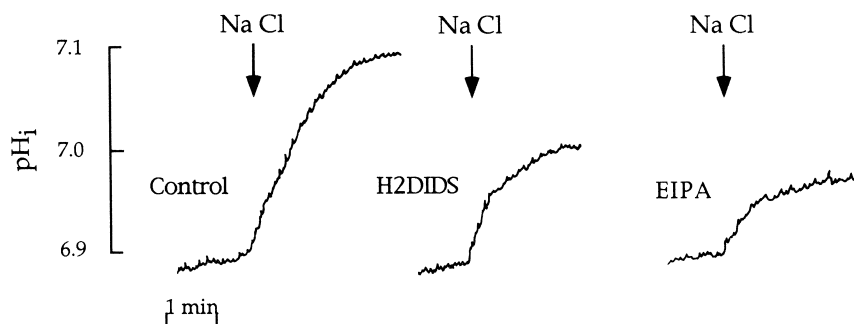


Fig. 4. pH_i recovery of acid loaded cells following addition of Na^+ to Na^+ -free solutions. All cells were acidified with the NH_4Cl technique in conjunction with the preincubation in Na^+ -free solutions in the presence (solution G) and absence (solution C) of bicarbonate. As a result pH_i was close to 6.88 in all series of experiments immediately prior to addition of $40 \text{ mmol l}^{-1} \text{ Na}^+$ (average pH_i value varied only between 6.87 ± 0.02 and 6.90 ± 0.02 in the individual series). When tested, the inhibitors were present in the ammonium prepulse. Following withdrawal of NH_4^+ cells were immediately suspended in Na^+ -free solutions containing the indicated inhibitors. The concentration of $\text{H}_2\text{-DIDS}$ was 0.5 mmol l^{-1} and that of EIPA $100 \mu\text{mol l}^{-1}$. At the time indicated by the arrow $40 \text{ mmol l}^{-1} \text{ NaCl}$ was added. Each trace is representative of five independent experiments.

recovery from a moderate acid load was totally dependent on the activity of the Na^+/H^+ exchanger, since the recovery was Na^+ -dependent, inhibited by

Table 5

Initial rate of pH_i recovery of acid-loaded cells following addition of Na^+ to Na^+ -free solutions

Conditions	Alkalinization on Na^+ readdition		
	dpH_i/dt (U min^{-1}) $\times 10^{-2}$	J_{H}^+ (nmol min^{-1} mg protein^{-1})	Inhibition %
<i>HEPES buffer</i>			
Control	12 ± 1	36.8 ± 3	
EIPA	$1 \pm 0.5^*$	$3 \pm 1.5^*$	92
<i>$\text{HCO}_3^-/\text{CO}_2$ buffer</i>			
Control	14 ± 1	$62 \pm 4^\#$	
EIPA	$4 \pm 0.5^*$	$18.3 \pm 2^*$	71
$\text{H}_2\text{-DIDS}$	$6.5 \pm 0.5^*$	$29.8 \pm 2^*$	50
<i>$\text{EIPA} + \text{H}_2\text{-DIDS}$</i>			
$\text{H}_2\text{-DIDS}$	$0.5 \pm 0.5^*$	$2.3 \pm 2.3^*$	100
<i>Cl^--free, $\text{HCO}_3^-/\text{CO}_2$ buffer</i>			
Control	13 ± 0.3	$59.6 \pm 1.4^\#$	
EIPA	$6 \pm 0.5^*$	$27.5 \pm 2^*$	46
$\text{H}_2\text{-DIDS}$	$6 \pm 0.5^*$	$27.5 \pm 2^*$	46

Net proton flux, J_{H}^+ , in $\text{nmol mg protein}^{-1} \text{ min}^{-1}$, was calculated as indicated in Section 2.

Inhibition refers to the observed J_{H}^+ value in the presence of inhibitor compared to respective control value of each series in absence of inhibitors.

Other details as in Fig. 4.

Means \pm S.E.M. of five independent determinations.

* $p < 0.001$, as compared with its own control (first row in each case).

$^\# p < 0.001$, as compared with values obtained in HEPES-buffered solution in the absence of modifiers (first row of the table).

EIPA and unaffected by $\text{H}_2\text{-DIDS}$. We have now repeated and extended these experiments.

The rate of Na^+ -dependent pH_i recovery from an acid load was monitored in either HEPES- or HCO_3^- -buffered solutions. As shown in Fig. 4 and Table 5, in the absence of bicarbonate the rate of pH_i recovery was suppressed by EIPA. Bicarbonate significantly increased the rate of Na^+ -dependent regulatory cell alkalinization and both, EIPA and $\text{H}_2\text{-DIDS}$ inhibited it and the effects of the inhibitors were additive.

In another set of experiments, cells were acidified in a nominally Na^+ - and Cl^- -free, bicarbonate-buffered solutions (solution H) following 20 min incubation in Cl^- -free, bicarbonate-buffered solutions (solution F). This procedure served to nominally deplete the cells of intracellular chloride. Cells were then placed in Na^+ and Cl^- -free solutions (solution H). The rates of Na^+ -dependent pH_i recovery were not different from those obtained in Cl^- -containing solutions (Table 5).

4. Discussion

The resting pH_i values of chicken colonocytes reported herein are lower than those reported for rabbit [9] and human [10] colonic crypt cells and for rabbit colonocytes [17], similar to those measured in the colon carcinoma cell line, HT₂₉, [7] and slightly more acidic in HCO_3^- than in HEPES-buffered solutions ($p < 0.01$).

Chicken colonocytes possess a Na^+/H^+ exchanger operative under resting conditions and responsible for pH_i recovery from an acid load in the nominal absence of HCO_3^- [11]. The current work presents evidence for the existence in the same cells of a Na^+ -independent $\text{Cl}^-/\text{HCO}_3^-$ exchanger and a $\text{Na}^+/\text{HCO}_3^-$ cotransporter.

The observations listed below are consistent with the presence of a Na^+ -independent $\text{Cl}^-/\text{HCO}_3^-$ exchanger which is involved in setting the resting pH_i and in pH_i recovery from an alkaline load: (i) intracellular alkalinization following external Cl^- removal, (ii) return to resting pH_i values upon reinstatement of Cl^- , (iii) recovery from alkaline loads required external Cl^- , and (iv) all these Cl^- -dependent processes were Na^+ -independent and inhibited by $\text{H}_2\text{-DIDS}$. The activity of the exchanger observed under resting conditions may be responsible for the slight decrease in pH_i due to external HCO_3^- . Although the involvement of $\text{Cl}^-/\text{HCO}_3^-$ exchanger in pH_i regulation is well established in several epithelial cell types (see Ref. [3] for review), there are only two reports for the large intestine. The exchanger regulates pH_i in HT29 cells [7], but not in the human colonic crypt cells [10].

The $\text{Cl}^-/\text{HCO}_3^-$ exchanger may have an absolute requirement for bicarbonate or only a preference for bicarbonate over OH^- (see Ref. [3] for review). The present results show that the Cl^- -dependent changes in pH_i occurred under HCO_3^- -free conditions, although at a lower rate than those observed in HCO_3^- -containing solutions. This indicates that, as demonstrated in apical membrane vesicles of rat distal colon [18] and in HT₂₉ cells [7], the $\text{Cl}^-/\text{HCO}_3^-$ exchanger of chicken colonocytes has not an absolute requirement for exogenous bicarbonate since it also permits transport of OH^- . Another reason for the observed Cl^- -dependent alkalinization under HCO_3^- -free conditions might be that metabolism produces sufficient endogenous $\text{CO}_2/\text{HCO}_3^-$ to support the slow activity of an exchanger without recourse to OH^- ions.

The set point of the $\text{Cl}^-/\text{HCO}_3^-$ exchanger (no net flux) of chicken colonocytes is a pH_i approx. 6.96, a value below the observed resting pH_i value. This observation agrees with those described above which suggest that the $\text{Cl}^-/\text{HCO}_3^-$ exchanger is functional under resting conditions. The relationship between net base efflux and pH_i shows that at resting pH_i the

$\text{Cl}^-/\text{HCO}_3^-$ exchanger functions at a low rate and its activity increases with increasing pH_i . This relationship has a Hill coefficient value greater than 1 ($n = 3.89$), indicating a positive cooperative mechanism for the influence of cytosolic base on $\text{Cl}^-/\text{HCO}_3^-$ exchanger. Similar observations have been reported for other cell types (see Ref. [3] for a review).

The current results also indicate that chicken colonocytes possess at least two Na^+ -dependent acid/base transporters operating during pH_i recovery from acid loads. One is the EIPA-sensitive Na^+/H^+ exchanger, which also functions under resting conditions, and the other is an EIPA-resistant mechanism. This latter pH_i regulatory mechanism has all the properties of the $\text{Na}^+/\text{HCO}_3^-$ cotransporter in that recovery was absolutely dependent on the presence of bicarbonate and Na^+ , it was inhibited by $\text{H}_2\text{-DIDS}$ and its rate of operation was unaffected by Cl^- -removal. This last criterion distinguishes between $\text{Na}^+/\text{HCO}_3^-$ cotransporter and Na^+ -dependent $\text{Cl}^-/\text{HCO}_3^-$ exchanger. Under resting conditions the $\text{Na}^+/\text{HCO}_3^-$ cotransporter does not appear to be functional, since the rate of Na^+ -induced pH_i changes was prevented by EIPA and unaffected neither by $\text{H}_2\text{-DIDS}$ nor by the presence of bicarbonate.

$\text{Na}^+/\text{HCO}_3^-$ cotransporter, originally described in the basolateral membrane of kidney proximal tubules in the tiger salamander [19], has later been described in a variety of other cells, including rat distal colon [20] and human colonic crypt cells [10]. In some cells the cotransporter is quiescent when assayed in nominally $\text{CO}_2/\text{HCO}_3^-$ -free solutions [21,22], but operates in other cells, apparently supported by metabolically generated HCO_3^- [23,24]. The $\text{Na}^+/\text{HCO}_3^-$ cotransporter present in chicken colonocytes has absolute requirement for exogenous bicarbonate, since it did not work at an appreciable rate in HEPES-buffered solutions.

Our study shows for the first time the presence of a $\text{Cl}^-/\text{HCO}_3^-$ exchanger and a $\text{Na}^+/\text{HCO}_3^-$ cotransporter in avian colonocytes. Among the two, only the $\text{Cl}^-/\text{HCO}_3^-$ appears to be functional under resting conditions and therefore, together with the Na^+/H^+ exchanger, participates in setting the resting pH_i . In addition the $\text{Cl}^-/\text{HCO}_3^-$ exchanger is involved in pH_i recovery from an alkaline load, whereas the $\text{Na}^+/\text{HCO}_3^-$ cotransporter plays a role in pH_i recovery from an acid load. The results offer no clues on

the cellular localization of the transporters. The $\text{Cl}^-/\text{HCO}_3^-$ exchanger is present in the brush border membrane, but not in the basolateral membrane, of rat colonocytes [18], whereas in HT₂₉ cells [25] the $\text{Cl}^-/\text{HCO}_3^-$ exchanger is localized in the basolateral membrane. The $\text{Na}^+/\text{HCO}_3^-$ cotransporter has been found in the basolateral membrane of rat distal colon [20]. In some cells the $\text{Na}^+/\text{HCO}_3^-$ cotransporter functions as a base loader and in others as an acid loader (see Ref. [3] for a review). Since we have not addressed the issue of electrogenicity and stoichiometry of the cotransporter we cannot say whether in vivo the cotransporter operates as a base loader or as a base extruder.

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